Melphalan and dexamethasone with or without bortezomib in newly diagnosed AL amyloidosis: A matched case–control study on 174 patients

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DOI: https://doi.org/10.1038/leu.2014.227

Leukemia (2014), 1–6

www.nature.com/leu

ORIGINAL ARTICLE

Melphalan and dexamethasone with or without bortezomib in newly diagnosed AL amyloidosis: a matched case–control study on 174 patients

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Oral melphalan and dexamethasone (MDex) is a standard treatment for patients with AL amyloidosis who are not eligible for stem cell transplantation at many referral centers. However, following encouraging reports on the activity of bortezomib combined with alkylators and dexamethasone, these combinations are being moved to frontline therapy. We compared the outcome of 87 patients treated with bortezomib plus MDex (BMDex) with that of 87 controls treated with MDex. Patients and controls were matched for age, cardiac and renal function and free light chain burden. A higher rate of complete responses was observed with BMDex (42 vs 19%), but this did not result in a survival improvement in the overall population. However, a significant survival advantage for BMDex was observed in patients without severe (New York Heart Association class III or IV) heart failure and with N-terminal pro-natriuretic peptide type-B < 8500 ng/l. Patients treated with full-dose dexamethasone had similar response rates and survival whether they received bortezomib or not. Intermediate-risk patients who are not fit enough to receive high-dose dexamethasone are likely to take the greatest advantage from the addition of bortezomib to MDex.

Leukemia advance online publication, 19 August 2014; doi:10.1038/leu.2014.227

INTRODUCTION

Immunoglobulin light chain (AL) amyloidosis is caused by a plasma cell clone, which is usually of a modest size, producing a light chain that misfolds and aggregates in the form of amyloid fibrils, causing systemic proteotoxicity.[1] The natural history of this disease is primarily determined by the severity of cardiac dysfunction.[2] Patients who present with advanced heart involvement, defined by very high levels of the cardiac biomarker N-terminal pro-natriuretic peptide type-B (NT-proBNP), survive only a few months and represent the most difficult challenge for treating physicians.[3] Clinical and experimental evidence indicate that cardiac damage in AL amyloidosis is mainly determined by a direct toxicity exerted by the circulating amyloidogenic free light chain (FLC).[4–8] Thus, therapy of AL amyloidosis is aimed at obtaining a rapid and profound FLC reduction by targeting the amyloidogenic plasma cell clone with chemotherapy, while attentively supporting involved organ function. Treatment approaches are derived from multiple myeloma and are adapted to the fragile amyloid patients, and novel agents are finding their place in the therapeutic armamentarium for AL amyloidosis.[9,10]

Bortezomib is now a mainstay in the treatment of multiple myeloma.[11] Treatment approaches based on proteasome inhibition are expected to be highly efficacious in AL amyloidosis, because amyloidogenic plasma cells rely on proteasome activity to deal with the proteotoxic stress caused by the misfolded light chain.[12–14] Two early series showed promising activity of bortezomib in AL amyloidosis, emphasizing its rapid action.[15,16] A prospective phase I/II clinical trial of bortezomib as a single agent showed a hematologic response in approximately two thirds of relapsed/refractory patients with AL amyloidosis.[17,18] In this trial, tolerability in patients with cardiac involvement was good, but subjects with advanced disease were excluded.[19] A large retrospective study including 94 patients, most of whom were relapsed or refractory after previous treatment, confirmed the efficacy of this drug in combination with dexamethasone (overall hematologic response rate 71%).[20] Moreover, bortezomib and dexamethasone can be used as 'adjuvant treatment' after autologous stem cell transplant (ASCT), improving the quality of response in almost 90% of cases who do not achieve complete response (CR) after transplantation.[21] More recently, high CR rates (42–71%) were reported in two small independent series of patients treated with the combination of cyclophosphamide, bortezomib and dexamethasone.[22,23] These encouraging results led to the perception that bortezomib-based treatment is superior to standard approaches for the treatment of AL amyloidosis, and this drug is increasingly prescribed outside the framework of clinical trials.

At our center, melphalan and dexamethasone (MDex)[24–26] was standard treatment for intermediate- and high-risk patients since 2004, whereas patients eligible for ASCT and those who have potentially reversible contraindications to ASCT are transplanted or treated with stem cell-sparing regimens.[27] The safety and efficacy profile of MDex in AL amyloidosis has been assessed in a randomized clinical trial and in several, independent series. The French multicenter trial showed no difference in response rate and survival between MDex and risk-adapted ASCT.[28] Despite the general good tolerability of MDex, the toxicity of high-dose dexamethasone in AL amyloidosis is not negligible, most common concerns being fluid retention and arrhythmias,[29,30] requiring dose reductions. Recently, we reported the results of a large

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Received 24 April 2014; revised 4 July 2014; accepted 15 July 2014; accepted article preview online 25 July 2014

retrospective study showing that hematologic response could be achieved in 76% (CR in 31%) of patients who were fit enough to receive MDex with full-dose dexamethasone, whereas only 51% (CR 12%) of patients responded to MDex with attenuated dexamethasone.[26] Other studies showed that MDex cannot overcome the poor prognosis of patients with severe cardiac involvement.[31] Based on the early reports of efficacy of bortezomib combined with alkylators and bortezomib, starting in 2007, we offered treatment with bortezomib associated with MDex (BMDex) to all the patients who had access to the drug and gave consent.

Currently, there is no study comparing novel agents combinations with standard approaches in AL amyloidosis, and the results of uncontrolled studies should be considered with great caution, because the outcome can be very diverse depending on different proportion of patients with advanced disease.[32,33] In 2011, a randomized clinical trial comparing MDex with BMDex was initiated (NCT01277016), which is still ongoing in Europe and Australia. However, the results of this trial will not be available for at least 2 years, although the increasing use of bortezomib combinations in routine clinical practice urgently requires some indications on the most appropriate setting to employ this drug in AL amyloidosis. Thus, we designed the present retrospective matched case–control study to assess whether the addition of bortezomib to MDex resulted in improved hematologic response rate and overall survival in 174 patients with AL amyloidosis.

PATIENTS AND METHODS

The whole study population comprises 174 matched patients with AL amyloidosis evaluated at our center between 2005 and 2012. The BMDex cohort is composed of all the 87 consecutive subjects treated with this regimen between 2007 and 2012. Bortezomib was added to standard MDex in all the patients who gave consent and to whom the drug could be prescribed according to Italian regulations. They were matched with 87 controls selected from a total of 335 subjects treated with MDex between 2005 and 2012. All the patients were newly diagnosed. The patients gave written informed consent as approved by institutional Ethics Committee. The amyloid deposits were characterized as AL-type by immunoelectron microscopy or proteomics in all cases.[34,35] Evidence of a monoclonal component of the same isotype of that identified in the amyloid fibrils at serum and urine immunofixation electrophoresis and/or an abnormal FLC κ/λ ratio was required.[36] Subjects with lytic bone lesions were excluded.

The patients were matched for age (5-year periods), presence of heart and renal involvement, Mayo Clinic cardiac stage,[37] NT-proBNP above or below 8500 ng/l (a marker of advanced cardiac involvement),[3] systolic blood pressure above or below 100 mm Hg,[3] treatment with full-dose (that is, 40 mg on days 1–4) dexamethasone,[26] estimated glomerular filtration rate above or below 30 ml/min per 1.73 m^2 and difference between involved (amyloidogenic) and uninvolved free light chain (dFLC) above or below 180 mg/l (this threshold having been recently incorporated in the revised Mayo Clinic staging system).[38] When more than one matched control was available, we chose the one whose date of diagnosis was closest to that of the BMDex patient.

All the patients started treatment within 1 month from diagnosis. All the patients received oral melphalan (0.22 mg/kg) and dexamethasone (40 mg/day) on days 1–4 in 28-day cycles. Bortezomib was added at the dosage of 1.3 mg/m^2 on days 1, 4, 8, and 11 in the BMDex cohort, and was administered intravenously. Patients with repetitive ventricular arrhythmias[39] and/or fluid retention $>3\%$ of body weight (referring to usual non-edematous body weight)[40,41] received attenuated MDex, with dexamethasone 20 mg/day.[26] Melphalan was reduced by 25% in patients with eGFR <30 ml/min per 1.73 m^2 (six subjects in both cohorts). The maximum allowed number of cycles was nine. Treatment was discontinued in case of toxicity, in the event a CR or any hematologic response plus organ response was obtained after cycle 6 or in case hematologic response was not reached by cycle 3. Patients who did not achieve at least partial response by cycle 3 were shifted to second-line therapy, as well as those who obtained unsatisfactory responses after cycle 6.

Hematologic response was assessed 6 months after treatment initiation according to the new consensus criteria of the International Society of Amyloidosis.[42] The analysis of response was by intent-to-treat, and the patients who died before the evaluation of response were considered non-responders. As organ responses can be delayed, we evaluated the best organ response obtained before shifting to second-line therapy.

Toxicity was assessed monthly until completion of treatment and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 (National Institutes of Health, Bethesda, MD, USA). As the criteria of organ (other than heart) progression and hematologic progression have not been updated since 2005, and considering that in AL amyloidosis organ dysfunction (particularly kidney damage) can progress despite hematologic response, we calculated and analyzed time to next therapy or death.[26,43]

Differences in variables between subgroups were evaluated by the Mann–Whitney of χ^2 tests, as appropriate. Survival curves were plotted according to Kaplan–Meier and differences in survival were evaluated for statistical significance with the log-rank test. Survival was calculated from the time of diagnosis. Moreover, a 6-month landmark analysis of survival was performed to exclude patients who died early and could have irreversible organ damage at diagnosis. MedCalc Statistical Software version 13.0.6 (MedCalc Software bvba, Ostend, Belgium) was used for computation.

RESULTS

A total of 87 patients treated with BMDex were matched with 87 controls who received MDex. The patients' characteristics are reported in Table 1. The only significant difference between the two cohorts was a higher proportion of patients with peripheral neuropathy in the MDex group (19 vs 6%). This was expected because patients with severe or painful neuropathy did not receive bortezomib owing to its potential neurotoxicity. There was no difference in the number of cycles performed in the two cohorts.

Fifteen patients (17%) experienced grade 3–4 adverse events in the MDex cohort and 19 (22%) in the BMDex cohort ($P=0.444$). Fluid retention and cytopenia were observed in 8 (9%) and 6 (7%) patients in each cohort. Peripheral neuropathy was observed in 4 (5%) BMDex patients and in none of the subjects who received MDex alone ($P=0.042$). One patient in the BMDex cohort developed renal failure during treatment and a deep venous thrombosis occurred in one MDex patient.

Response was assessed according to the revised criteria of the International Society of Amyloidosis.[42] Complete response required negative serum and urine immunofixation and normal FLC ratio, very good partial response was defined as a dFLC <40 mg/l, and partial response required a decrease of dFLC $>50\%$. Cardiac response or progression required a decrease or increase in N-terminal natriuretic peptide type-B (NT-proBNP) $>30\%$ and >300 ng/l. Baseline NT-proBNP had to be >650 ng/l to be evaluable. Renal response required a $>50\%$ decrease in proteinuria in the absence of a $\geqslant 25\%$ reduction in eGFR plus a $\geqslant 0.5$ mg/dl increase in serum creatinine.[44] Response to treatment is reported in Table 2. A higher proportion of CRs was observed in the BMDex cohort (42 vs 19%), and this resulted in a higher overall hematologic response rate (69 vs 51%). Importantly, when the analysis was restricted to the 23 patients treated with full-dose dexamethasone in each cohort, there was no significant difference in hematologic response rate (Table 2). The time to hematologic response could be calculated in 15 subjects, 6 receiving MDex and 9 receiving BMDex, who had monthly assessments of dFLC. All of them received full-dose dexamethasone. Median time to first response was 1.4 months (range 0.9–2.7 months) in the MDex group and 1.1 months (range 0.9–2.3 months) in the BMDex group. A cardiac response was achieved in 10 (13%) and 12 (16%) in the MDex and BMDex patients, respectively ($P=0.644$). A renal response was observed in 15 (27%) subjects in the MDex cohort and in 9 (16%) patients in the BMDex group ($P=0.166$). In the overall study population, the median time to cardiac response was 5.5 months (range 1.6–7.7 months) and median time to renal

Table 1. Patients' characteristics

Variables	*MDex (87 patients)* N *(%) or median (IQR)*	*BMDex (87 patients)* N *(%) or median (IQR)*	P
Age, years	69 (62–74)		Matched
Heart involvement	74 (85)		Matched
Renal involvement	55 (63)		Matched
Cardiac stage I/II/III	13 (15)/39 (45)/35 (40)		Matched
NT-proBNP >8500 ng/l	19 (22)		Matched
Systolic blood pressure <100 mm Hg	3 (3)		Matched
Full-dose dexamethasone	23 (26)		Matched
eGFR <30 ml/min	6 (7)		Matched
dFLC >180 mg/l	44 (51)		Matched
NYHA class III or IV	39 (45)	37 (42)	0.760
NT-proBNP, ng/l	3328 (998–8007)	2753 (1062–6706)	0.558
cTnI, ng/ml	0.064 (0.020–0.187)	0.079 (0.021–0.162)	0.877
Proteinuria, g/24 h	0.9 (0.3–4.1)	1.3 (0.64–6.3)	0.386
eGFR, ml/min per 1.73 m^2	69 (50–86)	71 (53–95)	0.288
Liver involvement	10 (11)	9 (10)	0.808
Peripheral nervous system involvement	17 (19)	5 (6)	0.006
Number of organs involved	2 (1–3)	2 (1–2)	0.202
dFLC, mg/l	185 (85–516)	199 (108–570)	0.734
Bone marrow plasma cell, %	12 (7–20)	13 (10–20)	0.216
Number of treatment cycles	3 (3–6)	4 (2–6)	0.428
Treated in 2005–2006	18 (21)	0 (0)	—
Treated in 2007–2009	41 (47)	19 (22)	<0.001
Treated in 2010–2012	28 (32)	68 (78)	<0.001

Abbreviations: BMDex, bortezomib, melphalan and dexamethasone; cTnI, cardiac troponin I; dFLC, difference between involved (amyloidogenic) and uninvolved circulating free light chain; eGFR, estimated glomerular filtration rate; IQR, interquartile range; MDex, melphalan plus dexamethasone; NT-proBNP, amino-terminal pro-natriuretic peptide type-B; NYHA, New York Heart Association.

Table 2. Hematologic response to treatment (by intent-to-treat)

Patient population	*Response category*	*MDex* N *(%)*	*BMDex* N *(%)*	P
Overall population (87 patients in each cohort)	Overall hematologic response	44 (51)	60 (69)	0.013
	CR	17 (19)	37 (42)	0.002
	VGPR	5 (6)	11 (13)	0.143
	PR	22 (26)	12 (14)	0.056
Patients treated with full-dose dexamethasone[a] (23 patients in each cohort)	Overall hematologic response	16 (70)	17 (74)	0.743
	CR	7 (30)	11 (48)	0.227
	VGPR	2 (9)	4 (17)	0.381
	PR	7 (30)	2 (9)	0.135

Abbreviations: BMDex, bortezomib, melphalan and dexamethasone; CR, complete response; MDex, melphalan plus dexamethasone; PR, partial response; VGPR, very good partial response. [a]Dexamethasone 40 mg on days 1–4.

response was 8.2 months (range 3.9–18.6 months) with no significant differences between the two cohorts.

The median follow-up of living patients was 26 months. Forty-eight patients (55%) died in the MDex cohort and 34 (39%) in the BMDex cohort. The proportion of patients dying in the first year after treatment initiation was similarly high in both groups (38% in the MDex cohort and 34% in the BMDex group). There was no significant difference in overall survival (Figure 1a) and in time to second-line therapy or death (Figure 1b) between the two cohorts. However, in a 6-month landmark analysis excluding patients who died early, subjects treated with BMDex had a significant survival advantage (Figure 2).

In the overall study population, New York Heart Association class III or IV heart failure and a NT-proBNP concentration >8500 ng/l were associated with very poor survival (median 9 vs 61 months, and 11 vs 64 months, respectively, $P < 0.001$). The patients with both risk factors had a median survival of only 3 months. The rate of cardiac response, however, was no different between patients with none (11 responders out of 62 patients evaluable for cardiac response, 18%) and one and/or two risk factors (11 responders out of 86 patients, 13%, $P = 0.403$). The addition of bortezomib did not improve the outcome of patients with severe heart failure (New York Heart Association class >III) and/or with NT-proBNP >8500 ng/l. However, subjects without these risk factors who received bortezomib survived significantly longer than those receiving MDex alone (Figure 3). Even in this group of patients, there was no significant survival advantage for BMDex, if full-dose dexamethasone could be used (15 patients in each group, 98 vs 86% surviving 2 years, with MDex and BMDex, respectively, $P = 0.965$). Also in the 6-month survival analysis, there was no significant benefit for the BMDex cohort in patients who received full-dose dexamethasone (median survival not reached in both groups, with 89% of patients surviving 2 years in the MDex group and 83% in the BMDex group, $P = 0.892$). As expected,

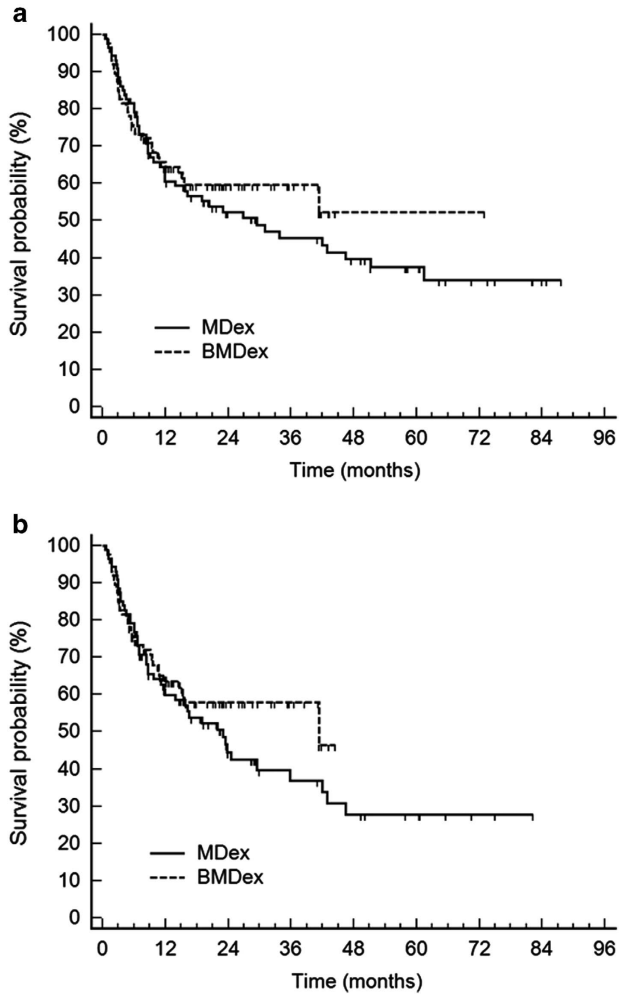

Figure 1. Survival according to treatment type. (**a**) Overall survival, median 30 months (95% confidence interval: 14–51 months) in the MDex cohort vs not reached (95% confidence interval: 19 months—not reached) in the BMDex cohort ($P=0.418$). (**b**) Time to second-line therapy or death, median 22 months (95% confidence interval: 12–36 months) in the MDex cohort vs 39 months (95% confidence interval: 15–41 months) in the BMDex cohort ($P=0.310$). BMDex, 87 cases; MDex, 87 matched controls.

survival was not affected by treatment type within each response category (that is, CR, very good partial response and partial response). The classical and revised Mayo Clinic staging system failed to identify a group of patients in whom treatment type was associated with a significant difference in survival.

In the MDex cohort, 35 patients received second-line therapy that was bortezomib plus dexamethasone in 18 cases, thalidomide based in 11 subjects and lenalidomide based in 6 patients. Hematologic response rate was 56% in bortezomib-treated patients. Three patients responded to lenalidomide, and one to thalidomide. In the BMDex cohort, 18 patients received rescue therapy that was lenalidomide based in 8 cases, thalidomide based in 5 cases and pomalidomide and dexamethasone in 5. Two patients responded to thalidomide, 3 to pomalidomide and 3 to lenalidomide. Interestingly, in the 6-month landmark analysis, the use of bortezomib upfront was still associated with a longer survival compared with sequential use of MDex and bortezomib (median 31 months vs not reached, $P=0.034$).

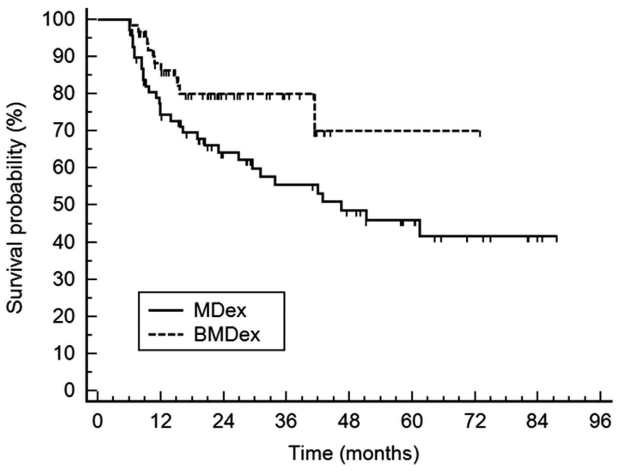

Figure 2. Six-month landmark analysis of survival according to treatment type. Median 47 months (95% confidence interval: 30–61 months) vs not reached (95% confidence interval: 41 months—not reached), $P=0.031$. BMDex, 63 patients; MDex, 69 patients.

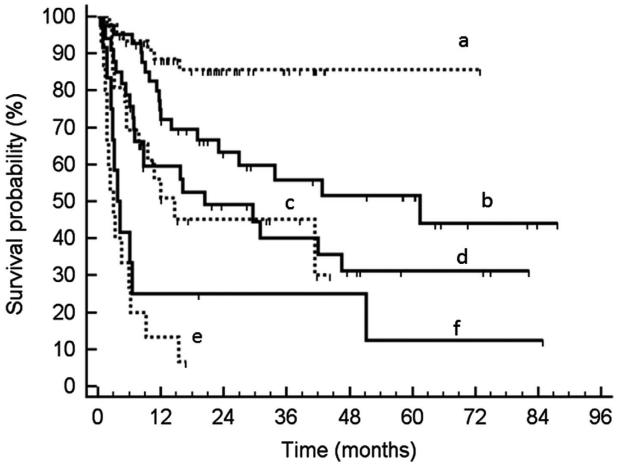

Figure 3. Survival according to NYHA class, NT-proBNP concentration and treatment type. Continuous lines indicate patients treated with MDex, dotted lines indicate subjects treated with BMDex. (a) NYHA class $<$ III and NT-proBNP $\leqslant$ 8500 ng/l treated with BMDex, 46 patients, median survival not reached (lower 95% confidence interval not reached; 86% surviving 2 years, 95% confidence interval 75–98%), $P=0.014$ compared with group b. (b) NYHA class $<$ III and NT-proBNP $\leqslant$ 8500 ng/l treated with MDex, 42 patients, median survival 61 months (95% confidence interval 23 months—not reached). (c) NYHA class $>$ III or NT-proBNP $>$ 8500 ng/l treated with BMDex, 26 patients, median survival 15 months (95% confidence interval 8–41 months), $P=0.783$ compared with group d. (d) NYHA class $>$ III or NT-proBNP $>$ 8500 ng/l treated with MDex, 33 patients, median survival 20 months (95% confidence interval 7–46 months). (e) NYHA class $>$ III and NT-proBNP $>$ 8500 ng/l treated with BMDex, 15 patients, median survival 3 months (95% confidence interval 2–6 months), $P=0.269$ compared with group f. (f) NYHA class $>$ III and NT-proBNP $>$ 8500 ng/l treated with BMDex, 12 patients, median survival 4 months (95% confidence interval 3–6 months). There was no significant difference in survival between patients treated with MDex and BMDex among subjects with NYHA class $>$ II or NT-proBNP $>$ 8500 ng/l.

DISCUSSION

Light chain amyloidosis is a very heterogeneous disease and the outcome is strictly dependent on the severity of organ damage,

particularly cardiac dysfunction, not only directly affecting patients' survival but also limiting the possibility of employing effective regimens.[9,45] In the present study, we analyzed two cohorts of patients with AL amyloidosis matched for all the most important disease-related prognostic factors. All the 87 patients diagnosed at our center and treated with BMDex frontline during the study period were included. The availability of a large population of patients treated with MDex (335 subjects) allowed for accurate matching. However, MDex patients were generally treated earlier than BMDex subjects, and had less options for rescue treatment.

Importantly, the study population was characterized by a remarkably elevated proportion of high-risk patients. Only one fourth of the subjects were deemed fit enough to receive full-dose dexamethasone, which is independently associated with higher response rates and prolonged survival.[26] Indeed, in our study, BMDex granted a lower rate of hematologic response compared with that reported by Gasparetto *et al.*[46] in the first prospective phase II trial of BMDex in AL amyloidosis (94%, with 56% CRs in 16 patients). This might be explained by the high proportion of patients with advanced disease receiving attenuated dexamethasone in our unselected series.

Although BMDex granted a significantly higher rate of CRs (42 vs 19%), this was not associated with improved overall survival, nor with longer time to second-line treatment or death in the overall population. With more than one third of patients dying in the first year after diagnosis in both cohorts, the presence of advanced, irreversible, cardiac dysfunction was the main determinant of survival. The addition of bortezomib could not overcome the poor prognosis of patients with severe heart involvement, identified by New York Heart Association class III and/or IV heart failure and/or NT-proBNP >8500 ng/l. This high concentration of NT-proBNP has been shown a marker of very poor outcome in AL amyloidosis in a large European study.[3] It is likely that the unsatisfactory cardiac response rate (13% with MDex and 16% with BMDex) is responsible for the dismal outcome of patients with advanced cardiac disease who can survive only if their cardiac damage improves. However, when subjects with severe heart involvement were excluded, a survival benefit associated with the addition of bortezomib appeared.

We observed that when full-dose dexamethasone was used, there was no difference in response rate, quality of response and survival between BMDex and MDex patients. However, this subgroup analysis was limited by the small number of patients and by the fact that these subjects were selected for less severe organ involvement, requiring a longer follow-up for survival differences to emerge.

Overall, our study showed that the outcome of patients with advanced cardiac dysfunction remains dismal even with BMDex, and the attempt to exploit the synergy of low-dose three-drug combination does not seem the conclusive answer for patients with advanced cardiac AL amyloidosis. Innovative strategies are urgently needed for these unfortunate patients. Our data suggest that 'intermediate-risk' patients, who do not have advanced heart failure at diagnosis, but still are not fit enough to receive full-dose dexamethasone, are those who are more likely to take advantage from the combination of BMDex. The small number of low-risk patients included in the present study and the relatively short follow-up prevent us to draw conclusions on the utility of adding bortezomib to MDex in these subjects. The ongoing phase III randomized and stratified clinical trial comparing full-dose BMDex and MDex (NCT01277016), will clarify the ideal setting for employing the combination of bortezomib, melphalan and dexamethasone in AL amyloidosis.

CONFLICT OF INTEREST

GM received honoraria from Millennium Takeda. The remaining authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by grants from 'Associazione Italiana per la Ricerca sul Cancro' Special Program Molecular Clinical Oncology 5 per mille n. 9965 'Harnessing tumor cell/microenvironment cross talk to treat mature B cell tumors, and from Cariplo Foundation (n. 2013-0964).

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